

WHAT IS CLAIMED IS:

1. A method for high-throughput amplification of one or more members of an oligonucleotide family which is related to a sample nucleic acid, said method comprising:
  1. growing a multiplicity of recombinant non-bacterial cell cultures containing an expression vector comprising at least one member of said oligonucleotide family whereby said member is transcribed and the number of copies of said oligonucleotide is amplified in each of said non-bacterial cell cultures.
2. The method according to Claim 1, wherein a transcription product of said one or more members of said oligonucleotide family is a ribozyme designed to cleave a target nucleic acid expressed by said non-bacterial cell cultures, wherein said target nucleic acid comprises a nucleotide sequence of said sample nucleic acid.
3. The method according to Claim 1, wherein a transcription product of said one or more members of said oligonucleotide family is an antisense nucleic acid designed to bind to a target nucleic acid expressed by said non-bacterial cell cultures, wherein said target nucleic acid comprises a nucleotide sequence of said sample nucleic acid.
4. The method according to Claim 1, wherein said recombinant non-bacterial cell cultures comprise mammalian cells.
5. The method according to Claim 1, wherein said expression vector is a plasmid or a virus.
6. The method according to Claim 5, wherein said virus is a retrovirus, or an adenovirus.
7. The method according to Claim 1, wherein said sample nucleic acid is a genomic DNA, a cDNA, an essential sequence tag (EST), or an RNA.
8. A method of assigning a function to a product coded for by a sample nucleotide sequence, said method comprising:
  1. growing a cell culture comprising one or more host cell(s) wherein said host cells express a target nucleic acid comprising said sample nucleotide sequence and wherein said

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host cells contain one or more members of a family of nucleic acids which bind to a transcription product of said nucleotide sequences whereby transcription product of said target nucleic acid is inhibited and said host cell exhibits at least one phenotypic change analyzing phenotypic changes in said cell to identify one or more altered function(s); and obtaining a nucleotide sequence of said target nucleic acid, whereby a function is assigned to said sample nucleotide sequences.

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9. The method according to Claim 8, wherein said function is a physiological

10 function.

10. The method according to Claim 8, wherein said function is enzyme activity.

11. The method according to Claim 8, wherein said function is protein synthesis.

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12. The method according to Claim 8, wherein said function is expression of a biological factor.

13. The method according to Claim 8, wherein said function is a regulatory effector  
20 function.

*Sub 13* 14. The method according to Claim 8, wherein said function is altered directly.

15. A double-stranded DNA comprising:

25 a sense strand and an antisense strand, wherein said sense strand codes for an antisense strand which when expressed as RNA binds to a mRNA sequence transcribed from a target nucleic acid sequence so that expression of a product of said target nucleic acid is inhibited, wherein a means for determining directionality of expression is included in said double-stranded DNA.

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16. The double-stranded DNA according to Claim 15, wherein said RNA comprises a catalytic domain which when expressed as RNA cleaves a mRNA sequence transcribed from

a target nucleic acid, and binding sequences flanking said catalytic domain for binding said RNA to said mRNA.

17. The double-stranded DNA according to Claim 16, wherein said RNA is a  
5 ribozyme.

18. The double-stranded DNA according to Claim 15, wherein said means for determining directionality of expression comprises a different non blunt-ended restriction enzyme site at each end of said double-stranded DNA.

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19. The double-stranded DNA according to Claim 18, wherein said double-stranded DNA is formed by contacting a first oligonucleotide with a complementary second oligonucleotide.

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20. The double-stranded DNA according to Claim 19, wherein said non blunt-ended restriction enzyme site is complementary to an end of said expression vector.

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21. A delivery vector comprising the double-stranded DNA according to Claim 15, wherein said delivery vector is formed by contacting a double-stranded oligonucleotide with  
an expression vector.

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22. A delivery vector comprising the double-stranded DNA according to Claim 15, wherein said delivery vector is formed by contacting a single-stranded oligonucleotide with an expression vector.

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23. A delivery vector comprising the double-stranded DNA according to Claim 15, wherein said double-stranded DNA is formed by contacting a triple-stranded oligonucleotide with an expression vector.

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24. The triple-stranded oligonucleotide according to Claim 23, wherein said triple-stranded oligonucleotide is formed by contacting a first oligonucleotide, a second oligonucleotide and a third oligonucleotide, wherein said second oligonucleotide is complementary to nucleotides at the 5' end of said first oligonucleotide, wherein said second

oligonucleotide further comprises excess nucleotides extending beyond the 5' end of said first oligonucleotide, wherein said third oligonucleotide is complementary to nucleotides at the 3' end of said first oligonucleotide, and wherein said third oligonucleotide contains excess nucleotides that extend beyond the 3' end of said first oligonucleotide.

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25. The triple-stranded oligonucleotide according to Claim 24, wherein said excess nucleotides are complementary to and base pair with the ends of said expression vector.

10 26. The double-stranded DNA according to Claim 22 or Claim 23, wherein said expression vector is filled in with Klenow.

27. The double-stranded DNA molecule according to Claim 21, Claim 22, or Claim 23, wherein said expression vector further comprises regulatory elements for expression.

15 28. A retrovirus expression vector comprising:

a retrovirus plasmid vector comprising a double-stranded DNA according to  
Claim 15.



20 29. The retrovirus expression vector according to Claim 28, wherein said vector comprises supercoiled DNA.

30. A retrovirus packaging cell line comprising:

a retrovirus expression vector according to Claim 28.

25 31. A retrovirus particle comprising:

a genome encoding expression of an RNA comprising a catalytic domain for cleavage of a mRNA transcribed from a target nucleic acid, and binding sequences flanking said catalytic domain for binding said RNA to said mRNA.

30 32. A mammalian cell comprising:

one or more double-stranded DNA(s) comprising a sense strand and an antisense strand, wherein said sense strand codes for a catalytic domain which when expressed as RNA cleaves a mRNA sequence transcribed from a target nucleic acid, and binding sequences

flanking said catalytic domain for binding said RNA to said mRNA, and wherein a means for determining directionality of expression is included in said one or more double-stranded DNA(s).

5       33. An adeno-associated virus expression vector comprising:  
an adeno-associated virus plasmid vector comprising a double-stranded DNA  
according to Claim 15.

10      34. An adeno-associated virus packaging cell line comprising:  
an adeno-associated virus plasmid vector according to Claim 33 and an adeno-  
associated virus helper plasmid.

15      35. An adeno-associated virus comprising:  
a genome encoding the expression of an RNA comprising a catalytic domain for  
cleavage of a mRNA transcribed from a target nucleic acid, and binding sequences flanking  
said catalytic domain for binding said RNA to said mRNA.

20      36. A plasmid expression vector comprising:  
a double-stranded DNA according to Claim 15.

25      37. A method for the introduction into a host cell(s) of a plasmid expression vector,  
said method comprising:  
contacting a cell culture comprising one or more host cell(s) with a co-precipitate of  
calcium phosphate and a double-stranded DNA comprising a sense strand and an antisense  
strand, wherein said sense strand codes for a catalytic domain, which when expressed as RNA  
cleaves a mRNA sequence transcribed from a target nucleic acid, and binding sequences  
flanking said catalytic domain for binding said RNA to said mRNA, and wherein a means for  
determining directionality of expression is included in said double-stranded DNA, whereby  
said plasmid expression vector is introduced into said host cells.

30      38. The method according to Claim 37, wherein said host cell(s) comprise  
mammalian cell(s).

39. The method according to Claim 37, wherein said plasmid expression vector is supercoiled DNA.

40. A method for expressing in a host cell a plasmid expression vector, said method 5 comprising:

introducing into said host cell said plasmid expression vector comprising a double-stranded DNA comprising a sense strand and an antisense strand, wherein said sense strand codes for a catalytic domain, which when expressed as RNA cleaves a mRNA sequence transcribed from a target nucleic acid, and binding sequences flanking said catalytic domain 10 for binding said RNA to said mRNA, and wherein a means for determining directionality of expression is included in said double-stranded DNA, whereby said plasmid expression vector is expressed in said host cells.

41. The method according to Claim 40, wherein said plasmid expression vector is 15 expressed in said host cell(s) without an intervening bacterial cloning step.

42. The method according to ~~Claim 40~~, wherein said plasmid expression vector is a retrovirus expression vector.

20 43. The method according to Claim 40, wherein said plasmid expression vector is an adeno-associated virus expression vector.

44. The method according to Claim 40, wherein said plasmid expression vector is contacted with gyrase.

25 45. A method for construction of a ribozyme vector, said method comprising: inserting a double-stranded DNA into a linearized delivery vector, wherein said double-stranded DNA comprises a sense strand and an antisense strand, wherein said sense strand codes for a catalytic domain which when expressed as RNA cleaves a mRNA sequence 30 transcribed from a target nucleic acid, and binding sequences flanking said catalytic domain for binding said RNA to said mRNA.

46. The method according to Claim 45, wherein said double-stranded DNA is formed by contacting a first oligonucleotide with a complementary second oligonucleotide.

47. The method according to Claim 46, wherein a means for determining directionality of expression is included in said double-stranded DNA.

5 48. The method according to Claim 47, wherein said means for determining directionality of expression comprises a different non blunt-ended restriction enzyme site at each end of said double-stranded DNA.

10 49. The method according to Claim 45, wherein said double-stranded DNA is formed by annealing a first oligonucleotide, a second oligonucleotide, and a third oligonucleotide, wherein said second oligonucleotide is complementary to nucleotides at the 5' end of said first oligonucleotide, wherein said second oligonucleotide further comprises excess nucleotides extending beyond the 5' end of said first oligonucleotide, wherein said third oligonucleotide is complementary to nucleotides at the 3' end of said first oligonucleotide, and wherein said 15 third oligonucleotide contains excess nucleotides that extend beyond the 3' end of said first oligonucleotide.

20 50. The method according to Claim 49, wherein said excess nucleotides are complementary to and base pair with the ends of said linearized delivery vector.

51. The method according to Claim 49, wherein said ribozyme vector is treated with a DNA polymerase.

25 52. The method according to Claim 51, wherein said DNA polymerase is a cellular DNA polymerase.

53. The method according to Claim 51, wherein said DNA polymerase is a Taq DNA polymerase.

30 54. The method according to Claim 51, wherein said DNA polymerase is Klenow.

55. A method for construction of a ribozyme vector, said method comprising:

contacting a single-stranded oligonucleotide with a linearized delivery vector wherein said single-stranded oligonucleotide or a strand that is complementary to said single-stranded oligonucleotide encodes a transcription product that is a ribozyme, wherein said single-stranded oligonucleotide is complementary to one end of said delivery vector and base pairs 5 with said delivery vector, and wherein said strand that is complementary to said single-stranded oligonucleotide is formed with a DNA polymerase.

56. The method according to ~~Claim 55~~, wherein said DNA polymerase is Klenow.

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57. The method according to ~~Claim 43 or Claim 55~~, wherein said ribozyme vector further comprises regulatory elements for expression.

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